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EXPERIMENTS ON SENSITIZATION

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EXPERIMENTS ON SENSITIZATION*

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EXPERIMENTS ON SENSITIZATION.

1. TECHNICS OF EXPERIMENT.

Guinea-pigs were sensitized with horse dander extract, and the sensitiveness of guinea-pigs was tested in the so-called DALE bath using intestinal segments.

a. The preparation of the horse dander extract.

The horse dander extract was prepared by COCA's method (1, 2, 3, 4.).

(1) Horse dander: The curryings (*fresh*) from clean horses are sifted through a domestic sieve into *water-free ether.*
The mixture of dander and ether is poured on a Büchner funnel over 2 layers of filter paper, and the dander washed with water-free ether under a moderate negative pressure until the runnings are almost colorless. From time to time, small portions of the dander are tested by shaking in a test tube with saline solution. A sufficient removal of fat is indicated when the dander can be emulsified easily in the aqueous fluid. After this point has been reached, the ether is removed by evaporation using a warm sand bath at 60–70°C. The dry powder, thus obtained is stored in a tightly sealed tin can.

(2) Extracting fluid: The extracting fluid was made by the modified method according to COCA. The 2 stock concentrated solutions are prepared as follows:

1.	KH_2PO_4	3·63 g
	$Na_2HPO_4 + 12H_2O$	14·31 „
	NaCl	50·00 „
	Distilled water up to	1 000·00 ccm
2.	Carbolic acid	4% solution.

Equal parts of these two stock solutions are mixed, one part of this mixture is added to 4 parts of distilled water. COCA recommended to order phosphate salts from the J. T. BAKER Chemical Co., Phillipsburg, New Jersey. Unfortunately, I could not get it from the company as the same had been out of stock when I ordered. Therefore, I used phosphate salts obtained from a chemical store and recrystalized it 3 times myself. The *p*H of the extracting fluid was 7·0. The fluid was autoclaved.

(3) Horse dander extract: 50 g of the dry powder of horse dander and 100 ccm of toluol were mixed and then adding 1 000 ccm of extracting fluid the same was mixed in a glass stoppered bottle.

The mixture was shaken in a machine for 3 hours, allowed to stand overnight at a room temperature, and the following morning the supernatant liquid was filtered by suction through a hardened paper. The filtrate was of slightly turbid fluid, which then was made to pass through a Berkefeld filter. The final fluid was crystal clear, and dark brown in color (5). It was desired to use this extract first in neutral reaction and the fluid was therefore brought to a *p*H of 7·4. As there was a tendency for the extract to become slightly alkaline if left standing, titrations were effected frequently, and when necessary the fluid was readjusted to a *p*H of 7·4. Horse dander extract was made twice during my experiments, in June 1928 and in January 1930. Chemical analysis done by Dr. K. M. JARVIS of Dr. COWIE's department is as follows:

	First extract,	Second extract,	LONGCOPE's preparation
Total nitrogen	50	56	48
N. P. N.	19	26	19
Protein nitrogen	31	30	29
Reducing sugar	13	26	24
*p*H	7·6	7·6	7·4

Culture on blood agar and in bouillon was negative. The extract was kept in an ice-box in rubber stoppered small glass bottles separately, each containing about 50 ccm for the sake of convenience.

b. Active sensitization.

Guinea-pigs weighing from 200 to 250 grams were selected and most scrupulous care was taken in regard to their physical conditions. During the period of experiment, they were fed with carrots, lettuce, oats, crashed wheat and timothy hay. They were put in a big clean pen with good bedding. The temperature of the room was kept constant, at about 65°F and the room was also well ventilated. The guinea-pigs were weighed at regular intervals. Normal increases of weight during the experiment was noticed. The guinea-pigs were sensitized with horse dander extract both by the parenteral and the enteral method.

(1) Parenteral method — Horse dander extract was injected intraperitoneally. A single injection of 1·0–2·5 ccm was found sufficient to produce a reaction in the bath when intestine is used, after an interval of more than 3 weeks.

(2) Oral method — Guinea-pigs were fed with horse dander extract by means of a pipette, dropping the solution into their mouths. One group was fed first with ox-bile 0·5 ccm, and about 30 minutes later, was given 0·5 ccm of horse dander extract. This was repeated daily in the morning before they were fed. Another group was fed only with horse dander extract, 0·5 ccm daily. After 2 weeks of daily feeding the administration was stopped, and more than 3 weeks after the first feeding of horse dander, they were used for the test. Ox-bile solution was made by adding distilled water to ox-bile powder in the proportion of 15 : 85 and by autoclaving the same.

c. Modified DALE-TYRODE bath, which is made adaptable to the intestinal strip method (6, 7.).

(1) Apparatus (Fig. 1). The TYRODE's solution in which the organ is suspended is contained in a beaker of 500 ccm. At the bottom of this beaker it is plugged with a rubber bung having one central

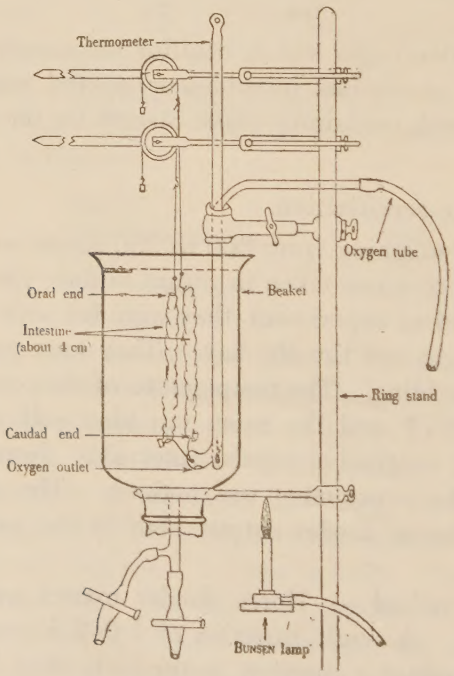

Fig. 1. Diagram of the bath (modified MAGNUS Apparatus).

bore. Through the latter passes one limb of a glass *T*-tube, which ends flush with the upper surface of the bung, so that the fluid in the beaker is enabled to run out through it to the last drop. When it is necessary to change the solution in the beaker during the test, a straight limb of the *T*-piece is connected by a rubber junction armed with spring clips to a waste pipe, through which the beaker is emptied. Another limb is connected to a syphon tube allowing the syphon to be filled with warmed TYRODE's solution immediately before a change is required. A beaker is held on a metal holder, the rod of which, a small BUNSEN burner heats, and by shifting this burner along the rod when necessary, the temperature of the solution can be kept easily between 38–39°C uniformly. Into the beaker dips a narrow glass tube 5 mm in diameter. This is turned at a right angle about half an inch above its lower end. The end is sealed with a blob; into this 3 small fish-hooks are fused. This serves to attach the lower end of the isolated organ. The upper end of the tube is connected to an oxygen tan through the rubber tube. A continuous stream of small oxygen bubbles thus rises through a hole and runs into the TYRODE's solution, a hole which is made at the lowest end of the glass tube on the opposite side of the angle, so that the bubbles do not play directly on the suspended organ. The size of the hole is *ca.* 2 mm in diameter, and oxygen bubbles are adjusted *at the rate of 30–40 per minute.* Thus it oxygenates and stirs the bath constantly throughout the test. The thermometer is put into the solution together with oxygen tube, tied by adhesive tape. The beaker is marked horizontally at the height of 300 ccm, after the thermometer and the oxygen tube are immersed, so that there will always be a right amount of solution in the bath. The upper end of the suspended organ is put on a small hook, which is made of an ordinary pin of half an inch long, by bending it. This is attached by a thin white silk thread to a junction from the pulley, which is made of thin aluminium. A thin white silk thread passes over pulley from the junction and is tied on the top of the pulley and is counterbalanced by a thin lead plate, weighing 0·17 grams. The strings can be shortened or lengthened at the junction to make the lever horizontal. The lever attached to the pulley is made of a thin celluloid paper, the point of which is cut sharply. The

ratio between lever arms magnifies the contraction (92; 16 = 5·7) 5·7 times, provided that there are no friction between lever point and paper and between axis of pulley and its holder. The lever point records its movement on the moving smoked paper of the kymograph. The kymograph moves constantly by electric motor, 43 mm per minute, recording on the paper at its bottom by a timer every 5 seconds. The ratio of magnification of contraction is rather great, and the counterbalance is less and the drum moves faster than in the experiments of rabbits' intestines by other workers. But this standard was arrived at after several tests were made and found that it seems to be fitted for the tender guinea-pig intestine. There is a microscrew on the holder of the pulley, so that it can be adjusted horizontally back and forth during the test. The bath can be operated with 3 segments at a time, putting one pulley above each. The horse dander extract is added to the bath by means of a syringe and needle by dropping it on the surface of the bath at the corner where the oxygen tube is immersed. The whole apparatus is set on a large table and any draft in the room or any vibration of the table is avoided. After each test, the oxygen tube and the beaker are carefully washed especially when the horse dander extract was added to the bath. The silk thread of the hook is renewed at every test.

(2) TYRODE's solution (8); The solution is made up on the following formula.

The first solution.		The second solution.	
NaCl	8·0	NaH_2Po_4	0·05
KCl	0·2	$NaHCO_3$	1·0
$CaCl_2$	0·2	Distilled water up to 200 ccm.	
$MgCl_2$	0·1		
Distilled water up to 800 ccm.			

Shortly before use, the first and second solutions are mixed, and to which 1·0 gram of glucose is added.

(3) The method of excision and suspension of the intestinal strip: Guinea-pig is killed by a blow on its head, at the base of the skull. The abdomen is widely opened. The duodenum is detached at its ascending part from the stomach and the descending colon from the rectum. Then the mesenterium is cut at the fixing points to the body wall and a whole mass of intestine is removed

to a large dish which contains warm TYRODE solution (38°C). Greatest care is taken to prevent shock to tissues, which as ALVAREZ (11) showed, is occasioned by rough handling. Pieces from 10–20 cm in length are removed from various parts of the intestine (duodenum, the middle part or the lower part of the ileum and from the colon). The lower part of the ileum is always cut a few inches up from its caecal end. The middle part of the ileum is selected where it contains less feces. After the removal of segments, the circulatory contraction is visible and it pushes out the contents of the segments. Therefore it is not necessary to wash out the intestinal contents artificially, but is only required to wait for a few minutes. These segments can be kept in fresh TYRODE solution and smaller pieces are cut off as required when the segments are in a relaxed condition. For the overnight test, they are kept in an ice-box, in fresh TYRODE solution. The segments of the intestine for the test are all cut in the same length, about 4–5 cm long, and the mesenterium is dissected away. The lower (caudad) end of the segment is attached to a glass supporting rod and the upper (orad) end of the segment is attached by a metal hook to a thread. The orad end of the segment can be determined as follows; 1. There is a tendency for a loop of the guinea-pig intestine to show a greater contraction at the orad than at the caudad end (TRENDELENBURG 9, 10), and peristalsis starts from the orad to the caudad direction. 2. The cuffing (the cut ends roll over so as to form little cuffs) is greater at the proximal than at the distal end of the segment (ALVAREZ 11). After the strip is fixed, the oxygen tube is then lowered into the beaker of warm TYRODE's solution (38°C). One must make it sure that the thermometer is not touching the beaker at its bottom. The intestinal strip between the 2 hooks in the bath, looks like a thin ribbon hanging. Usually it has no solid content. For the first 10–15 minutes after the strips are fixed, the contraction is very irregular. The circular muscle contraction is visible, especially in the ileum. Also there will be seen a change in tonus so that it is necessary to readjust the strings to bringhe t levers horizontal. Therefore, it is advisable not to start the kymograph until after this irritable stage is over.

(4) Special attention required when old intestinal segments are used:

When the intestinal strips are kept in an ice-box overnight, special attention is necessary to bring the solution from the freezing point up to the body temperature. It is first taken out from the ice-box and is allowed to stand for about 30 minutes to bring it up to the room temperature. The solution is then replaced with fresh TYRODE and is gradually brought to 38°C in a warm water bath. After the strips are set in the bath, one waits for about 30 minutes, because the tonus of the musculature increases and the strips shorten to 3/4 of their first length. (The upper part of the intestine shortens more than the lower part). The ability of rhythmic contraction returns to the musculature gradually.

d. Apparatus for the circular muscle contraction (Fig. 2).

This apparatus is the COWIE-LASHMET modification (12) of the TRENDELENBURG's apparatus. Longitudinal contraction is recorded just the same as with the MAGNUS apparatus. Circular muscle contraction is recorded by air displacement through a glass tube to a tambour (*T*). Tension on the inside of the gut could be increased or decreased at will by increasing or decreasing the hydrostatic pressure.

The intestinal strip is tied at both ends so that the pressure will not leak when it is increased. Thus one end is sealed up, so the effect of hydrostatic pressure is instant. First fill up the pressure tube with warm TYRODE's solution, then fix the intestinal strip and bring the level of the reservoir to zero on the scale, so that the head at *A* of the strip is at zero. Move the pressure bottle up and the height on the scale shows the hydrostatic pressure at *A*.

The bottle should be moved very slowly, 1 mm in 2 to 4 seconds, as pointed out by TRENDELENBURG. As we see in the diagram, the head (pressure) at *A* (caudad end) is higher than at *B* (orad end), *i. e.* caudad end receives larger pressure than orad end. The volume of the pressure bottle should be large enough so that the change of level in the bottle due to air pressure in the glass tube will be the minimum.

e. Discussion on the method.

(1) Antigenic properties of the horse dander: Though fewer experiments have been reported upon active anaphylaxis with extracts of animal dander, the results have been somewhat more conflicting than those obtained with extracts of pollen.

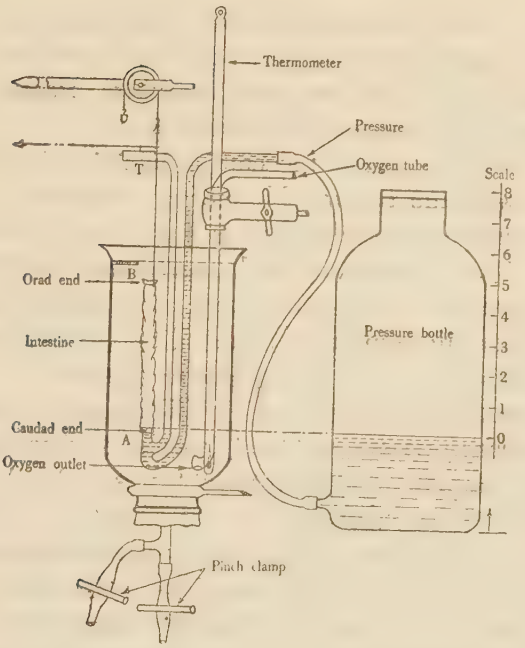

Fig. 2. Diagram of the bath (COWIE-LASHMET modification of TRENDELENBURG Apparatus).

COOKE, FLOOD and COCA (14) give protocols which show that guinea-pigs could be actively sensitized by extracts of horse epithelium (sensitizing dose is 0·5 ccm subcutaneously, minimum lethal dose is 0·1 ccm intravenously). They report that guinea-pigs sensitized by horse serum are not sensitive to horse dander, and the guinea-pigs sensitized by horse epithelium show no symptoms when injected intravenously with horse serum of shock producing dose.

FRIEDBERGER and KAMIO (15) conclude that guinea-pigs can not for certainty be sensitized by horse dander extract, a result which does not uphold the view that anaphylaxis in guinea-pigs and hypersensitiveness to horse dander in man are in identical conditions. They employed the COCA extract of horse dander, and repeated the experiments.

BUSSON and OGATA (16) have controverted the conclusions of FRIEDBERGER and KAMIO. They were able to sensitize guinea-pigs by an extract of horse dander. Sensitization could be accom-

plished by intraperitoneal injections and by inhalation of the extract, and anaphylactic shock could be produced both by intravenous injection and intranasal spraying. Desensitization could not be accomplished by intranasal spraying.

WALKER (17) studied the complement fixation and precipitin reactions with the serum of bronchial asthmatics. Complement fixation reactions were done using 3 proteins of horse dandruff as antigens, *i. e.* peptone, the alkali metaprotein and the coagulated protein were used. With the serum of 11 patients with bronchial asthma (they are associated, some of the attacks, with horses), 3 gave positive fixation, 8 gave negative fixation.

REMIREZ (18) reports a case in which passive transfer of hypersensitiveness to horses by blood transfusion from an asthmatic to horse to a normal man (35 years old) was realized. (2 weeks after receiving 600 ccm of blood.)

LONGCOPE, O'BRIEN and PERLZWEIG (5) have shown definitely that guinea-pigs can be made highly sensitive to extracts of horse dander, for sensitized guinea-pigs die in acute anaphylactic shock when injected intravenously with this extract, and the uteri of sensitized virgin guinea-pigs respond and contract in a characteristic manner on the addition of small quantities of extract to the DALE bath. Guinea-pigs sensitized by horse dander extract may be desensitized by this extract. The sensitization of guinea-pigs brought about by the injection of horse dander extact is specific.

It is possible to conclude, therefore, as LONGCOPE stated, from these experiments that extracts of horse dander contain an antigen or antigens which give anaphylactic reactions in guinea-pigs that are precisely the same as the anaphylactic reactions produced by any one of the large number of proteins.

(2) Solution for the DALE-bath: The research in the mode of action of some purgative salts was performed by TYRODE (8) with the isolated loop of a rabbit's intestine. He experimented with different kinds of physiological solution such as RINGER's, LOCKE's, HEDON's and ADLER's in regard to the study of the irritability of the intestine. He gave a table showing the composition of his nutritive fluid as compared with the others which he tried.

Artificial nourishing fluids:

	Ringer	Locke	Hedon	Adler	Tyrode
NaCl	6·0	9·0	6·0	5·9	8·0 g
KCl	0·05	0·25	0·3	0·4	0·2
$CaCl_2$		0·25	0·1	0·4	0·2
$MgCl_2$			($MgSO_4$ 0·3)	0·25	0·05
NaH_2PO_4			0·5	0·126	0·05
$NaHCO_3$	0·1	0·2	1·5	3·51	1·0
Glucose		1·0	1·0	1·5	1·0
Oxygen	Saturated		Sat.	Sat.	Sat.
H_2O	1 000·0	1 000·0	1 000·0	1 000·0	1 000·0 ccm

Tyrode's solution has proved an extremely effective medium for preserving the excitability of the isolated guinea-pig's uterus, without having a spontaneous development of high tonus or a large rhythm. Magnesium appears to possess a marked power of restraining the development of irregular automatic rhythm as Dale cited it.

Neukirch (19) used Tyrode's solution in his experiment with the isolated rabbit's intestine and noticed that Tyrode's solution has a great advantage over Ringer's solution in preserving the regular rhythm and normal excitability.

(3) The enteral method of sensitization, using bile solution: In studying alimentary anaphylaxis and the particular permeability of the intestinal canal, Makaroff experimented on guinea-pig (20). In the case of adult guinea-pigs, the sensitization by mouth being difficult, he tried to make the intestine permeable by means of bile and he succeeded in producing the anaphylactic state by adding milk to bile even in the case of guinea-pigs weighing 500 grams or more. He concludes as follows: 1. Alimentary anaphylaxis can be induced to the animals which take food that is not their habitual diet in large doses. It is easy to induce it to the younger animals. 2. Adult animals lend themselves to oral sensitization if one pays attention to increasing the permeability of the intestine by previous administration of bile. 3. Anaphylaxis induced by ingestion does not differ with regard to its characters from that induced by the injection of these substances.

After his experiments, Arloing, Langeron and Spassitch (21) worked together, using various kinds of protein mixed with bile,

and proved the constant alimentary sensitization by this method. They agree that the heterologous bile seems almost indispensable for the production of the phenomenon. They also found the absence of narrow specificity of the digestive anaphylaxis, *i. e.* they obtained the positive shock either with the same antigen or different ones. This multiplicity is analogous to lack of specificity of hypersensitiveness to food among human beings (28, 29, 30.). They also mention the constancy of shock producing by enteral administration of antigen in animals sensitized either by the enteral or the parenteral method.

(4) The intestinal strip method: HAFFTER (22) seems to have been the first who noticed that the intestinal strips which are taken out of the body could still continue to contract rhythmically for a while. OTTO and COHNHEIM (23) found that cat's intestine in oxygenated blood contracted itself lively for many hours. MAGNUS (13) studied on the excised segment of the intestine most in detail and standardized the method for the study of the excised segments, which now goes by his name. ALVAREZ (11) modified MAGNUS method and observed the important phenomenon of the intestinal gradients among the segments from different parts. The intestinal strip method depends upon autonony of the muscle in rhythmical condition, even in the absence of nervous stimuli (The myogenic nature of the rhythmic contractions). It is most advisable to read ALVAREZ's book (11) on this subject.

A preference for the intestinal strip method in sensitization: DALE (6, 7.) says that he has always found the intestinal muscle far less specifically sensitive than the uterus, and states that in regularity and delicacy of response no organ can approach a uterine horn from a virgin guinea-pig. However, if we could prove the specificity of the intestinal strip method in sensitization, then we might also have a preference for an intestinal strip method. In the uterine method the animals to be experimented should be virgin guinea-pigs of less than 250 grams in weight. In the intestinal method no consideration used to be paid as to the weight nor the sex of the animal. In the case of the former method there is only the possibility of a double check on account of there being only 2 uterus horns. On the other hand, with the intestinal strip method, we may use as many as we want for checking. If we

can assume that the sensibility to the antigen of the sensitized intestine is retained as long as it retains the power of rhythmic contraction, then the intestine can be used for the sensitization test at least 24–48 hours after the animal is killed. In the intestinal method, using a guinea-pig, besides the observation on the sensitization test, there can be seen many interesting physiological signs of its function, *which are more or less analogous to those prevailing in human beings.*

(5) Sensitiveness of isolated smooth muscle reactions in sensitization test: The DALE method of testing is a more accurate and finer indicator of the presence of specific antibodies than any other method. Smooth muscle reactions recorded are not always reliable indexes of the anaphylactic potentiality of the animal as a whole. For instance, non-specific desensitization of the entire animal does not always destroy the anaphylactic potentiality of the isolated smooth muscle. Even the uterus removed from an actively sensitized guinea-pig which has been killed in anaphylactic shock still may give a somewhat impaired response to antigen put into the bath (WEIL, 24). However, desensitization of the passively sensitized guinea-pig deprives the uterus of its power of response. The gradual and spontaneous loss of sensitiveness of the passively prepared guinea-pig is accompanied by a loss on the part of the uterus in its capacity to respond to antigen (WEIL, 24). Applying the sensitiveness of the isolated smooth muscle reaction, the following experiment would also be suggested. Sensitize a guinea-pig passively with the blood of hayfever, food-allergy, or asthma-patients, (COWIE and SCHLOSS, 30) and using the intestinal strip method, add the supposed antigens to the bath, those which are recognized by the scratch and intradermal test by patients. One may find the antigenic properties by the shock reaction of the sensitized intestine, to which the patient is sensitive and which is one of the causes of the disease. On this assumption, I worked in CZERNY's clinic in Berlin under Prof. HAMBURGER's approval, with children suffering from eczema.

2. INTESTINAL GRADIENTS IN NORMAL AND SENSITIZED GUINEA-PIGS.

a. Gradients of frequency (rate) and of amplitude of the pendulum movements in normal and sensitized guinea-pigs.

This experiment will be summarized in the following charts.

Chart I. The gradient of rate of contraction.
- a. In normal guinea-pigs.
- b. In sensitized guinea-pigs.
- c. Comparison between normal and sensitized guinea-pigs.

Chart II. The gradient of amplitude of contraction.
- a. In normal guinea-pigs.
- b. In sensitized guinea-pigs.
- c. Comparison between normal and sensitized guinea-pigs.

In these charts, the curves for the normal intestine represent the average of 11 guinea-pigs; for the sensitized intestine the average of 22 guinea-pigs. The charts were constructed in the following manner. Frequency was determined by counting the number of contractions in one minute, and the amplitude was determined for the same period of time by the average of the upstroke of the contractions measured in millimeters.

Approximately ten such counts for the frequency and amplitude of contraction were calculated from typical portions of each guinea-pig's kymograph record. These were plotted against time in the individual curves on graph paper, the ordinates representing the frequency (Chart I) and the amplitude (Chart II) of contractions and the abscissae the time in minute of the progress of the record. From these curves, an average of all the observations for every two minutes was calculated and plotted and then the final smooth curve was drawn through these plotted points.

In getting a curve as above, it is based upon the assumption that the intestine from each animal was treated in the same way with regard to all its environmental factors and stimuli. As greatest care was taken in this respect, only 33 intestines out of 60 guinea-pigs could be used for the analysis.

The gradient of rate of contraction: In chart I-a, we can see a gradient of rate of contraction in normal guinea-pigs. During the period between the 15th and 35th minute from the start, one can get a clear idea of gradient of rate in the normal intestine, *i. e.* the duodenum about 37 in a minute; the middle ileum about 36 in a minute; the lower ileum about 33 in a minute:

At the beginning of tests, the rate of the duodenal and the middle ileal segments is gradually increased, while that of the lower ileal

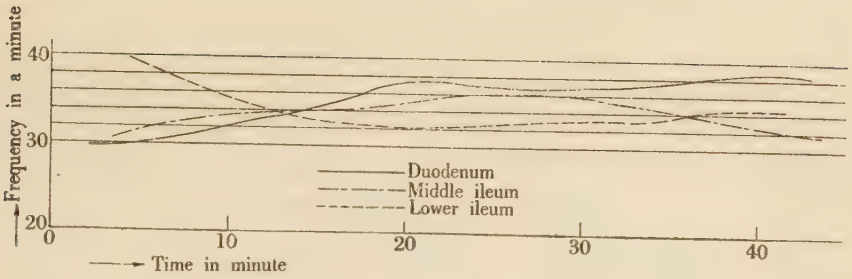

Chart I-a Normal intestine.

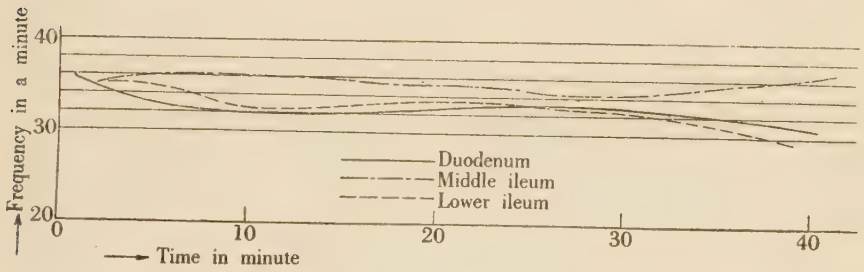

Chart I-b Sensitized intestine.

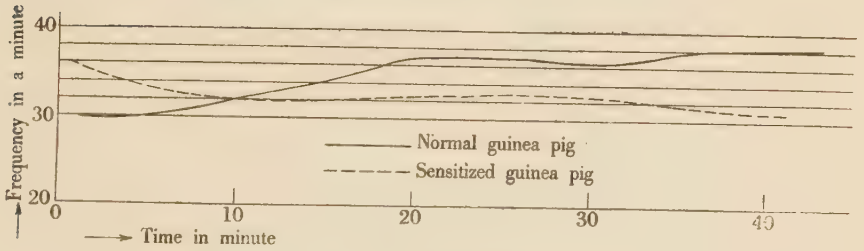

Chart I-c Duodenum.

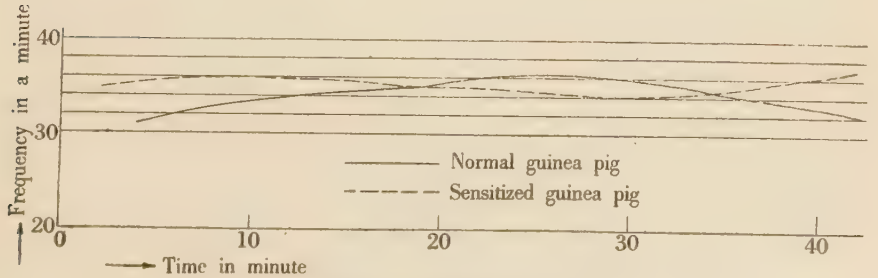

Chart I-c Middle ileum.

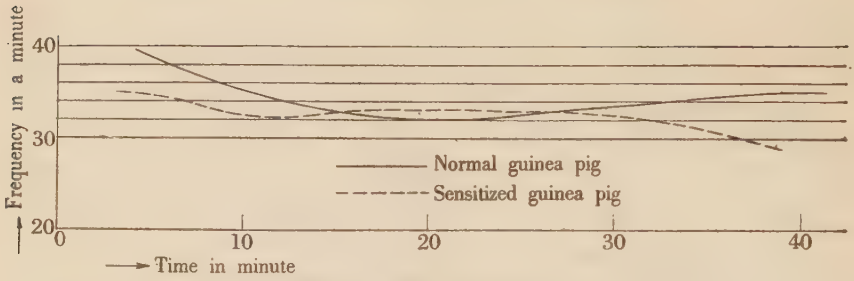

Chart I-c Lower ileum.

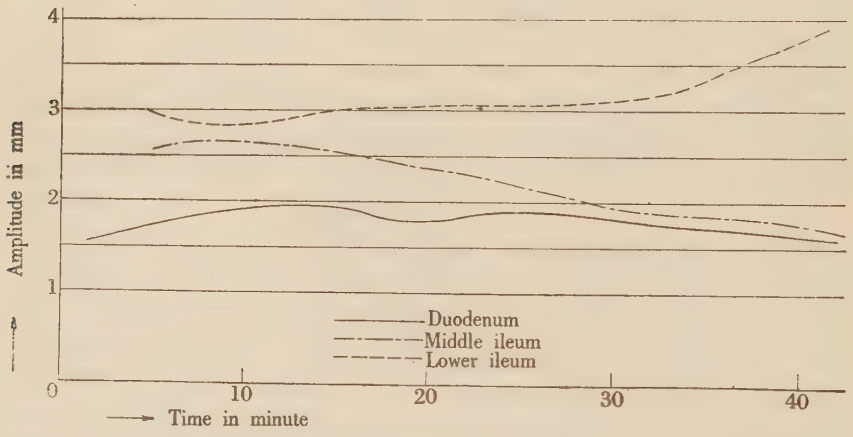

Chart II-a Normal intestine.

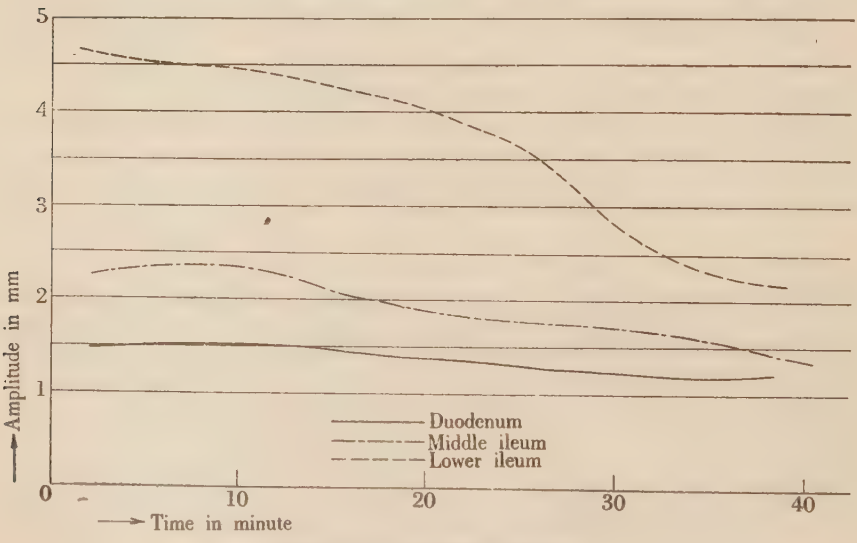

Chart II-b Sensitized intestine.

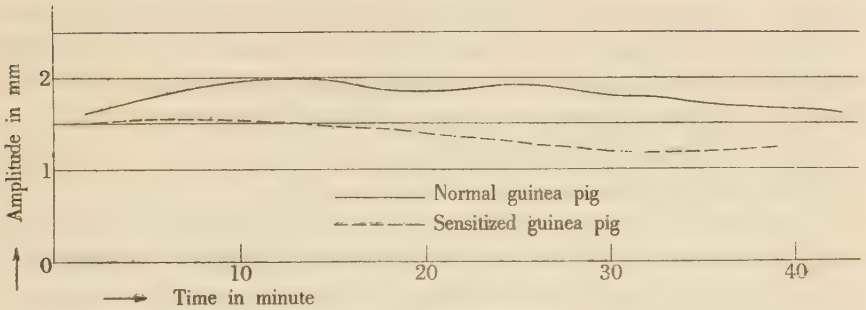

Chart II-c Duodenum.

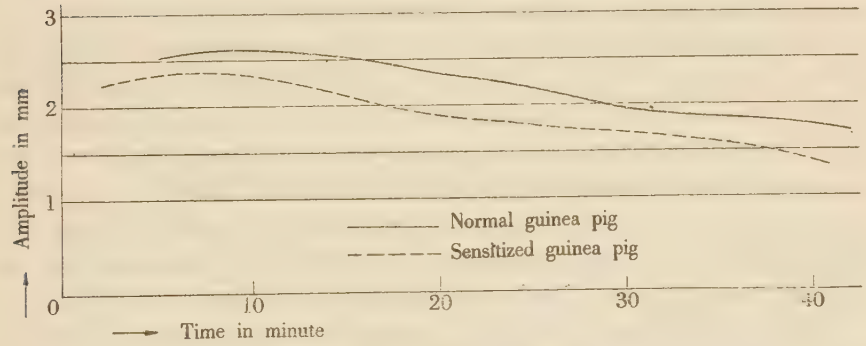

Chart II-c Middle ileum.

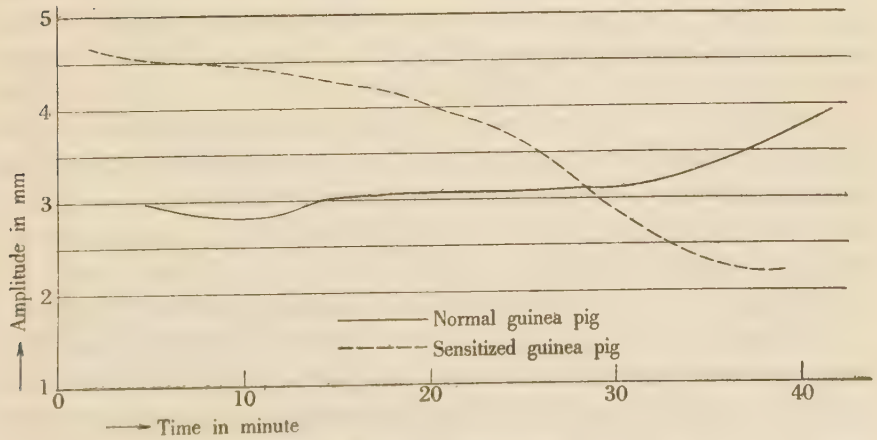

Chart II-c Lower ileum.

segments is decreased. This I believe demonstrates that the tonus gradient is refractory at this time. By the removal of the intestine from the body, it receives a great deal of unordinary stimulus. Since the lower part of the intestine has less tonus, it is more or less in a irritated condition at the beginning of the experiment. This point has been carefully considered by ALVAREZ (11).

Comparing chart I-b to chart I-a, in the sensitized intestine, the gradient of rate which we see in the normal intestine is somewhat changed and is rather in a labil state even in the period between the 15th and 35th minute, and the rate is the duodenum—about 32 in a minute; the middle ileum—about 35 in a minute; the lower ileum about 33 in a minute. For the test of sensitization, one gets always a good result at 10–15 minutes after the start.

In chart 1-c, the contrast of the curves between the normal and the sensitized guinea-pigs is shown. It will be seen that during the period of from the 20 th to the 30 th minute, the normal duodenum and the middle ileum have a greater frequency than the sensitized ones, while in the lower ileum the rate is about the same. In general, however, the difference in frequency between the normal and the sensitized intestines is rather small, and it becomes less marked as we go further down from the duodenum to the ileum.

The gradient of amplitude of contraction: In the normal guinea-pig's intestine (Chart II-a) we see the gradient of amplitude, *i. e.* the duodenum has an amplitude of less than 2 mm, the middle ileum more than 2 mm, and the lower ileum about 3 mm. In the sensitized intestine (Chart II-b), the duodenum has about one and a half and the middle ileum two and the lower ileum four mm amplitude. Thus 3 curves show a larger disparity and the tonus is more labil than the normal intestine. Contrasting the curves of amplitude of the normal and the sensitized intestines in chart II-c, the sensitized intestine has lower amplitude in the duodenum and in the middle ileum than that of the normal, while the lower ileum of the sensitized has a higher amplitude than that of the normal. Here is an analogy with the curves of rate in relationship between the normal and the sensitized intestines.

When we observe both gradients of rate and amplitude together, in the normal intestine, the duodenum has a lower amplitude at a faster rate than the lower part of the intestine has and as it goes caudad, there will be seen a higher amplitude with less frequency. In the

sensitized intestine, this normal correlation of the rate and amplitude is somewhat affected, *i. e.* the sensitized duodenum has a lower amplitude and less frequency than the normal. Consequently it gives a still larger difference than when we contrast the rate and amplitude separately. The middle and the lower ileum give also a little different view from the normal; the former has less frequency with the same amplitude and the latter has a higher amplitude with the same frequency when compared with the normal. Now contrasting the gradients of rate and amplitude of the normal and the sensitized intestine, I may draw the following conclusions. 1. Does it show that the sensitized guinea-pig has a smaller tonus in the upper part of the small intestine than the normal one? 2. Does it show that the sensitized guinea-pig is affected more in the upper part than the lower part of the small instestine? 3. If so, would it favour reverse peristalsis? (Would it rather suggest vomiting or diarrhea to patients of food allergy against sensitive foods?)

b. Gradient of latent period, by the influence of antigen.

By "latent period" I mean the time elapsed after antigen (horse dander) is put in the bath till the intestinal strips start to contract in a typically spastic manner (shock). The time is marked on the smoked paper at the beginning of dropping antigen into the bath. Usually, it takes about 10 seconds to drop 0·1 ccm of the horse dander extract from a syringe needle. The stirring action of oxygen bubbles is very small when they come out from a little hole at the rate of 30–40 per minute. The oxygen bubbles should be regulated constantly, so that antigen which is dropped on the surface of the bath is always mixed at about the same speed. When the horse dander is brought into contact with the intestinal strip, it has to reach the muscle fibres through the peritoneal covering and connective tissue (except the raw end). Thus, the latent period in its real sense, *i. e.* the time between the stimulus directly to the muscle and its response, is quite short.

The latent period in 24 fresh intestines and 10 old intestines is recorded for the purpose of showing the gradient of latent period (Table II). It will be seen that the latent period shows a definite gradation from a long latent period in the duodenal segments to a short one in the lower ileal segments. To this finding, there are exceptions in 5 cases; Nos. 472, 949, 480, 982 and 979 in part B. However,

we can overlook these cases, because No. 949 received a very small amount of antigen and Nos. 472, 982 and 979 have little difference in the time.

ALVAREZ observes that the latent period (in the ordinary sense) depends largely on the metabolic rate of the muscle. As the metabolic rate has been shown to vary in different parts of gut, we should expect the latent period to vary. He has shown a short latent period in the lower duodenum or the upper jejunum gradually lengthening to a long one in the lower ileum.

In the sensitized guinea-pigs intestines, I found that the latent period *i. e.* the time between the introduction of antigen into the bath and the anaphylactic muscle response is the reverse of that found in normal guinea-pigs by ALVAREZ. The gradient of latent period in anaphylactic response will be discussed in relation with the tonus gradient. Since the tonus of segments from the lower part of the small intestine is low, they react on a small stimulus at a quick response. In the duodenal segments, the tonus is higher and a stronger stimulus is needed to bring about a response. In other words, a larger amount of antibody in the segments must be supplied to antigen, and the physiological equilibrium in the cell must be disturbed more to result in a shock reaction than it is necessary in tissues of less marked in tonus, such as is found in the lower segments. Guinea-pig No. 985 showed the reaction only in the lower ileum, and not in the duodenum. In this case, I suppose the amount of antigen in the bath was not sufficient to produce shock in the duodenum. However, I have noted that if the duodenal segments react to antigen, they quickly reach their maximum point of contraction and thus steeper curves result than when the lower segments react. This time relationship among various segments of the intestine is shown in Table II. An increasing gradation in time from the duodenal segments to the lower ileal segments is definitely observed.

The maximum height of contraction is measured in millimeter from the base line, vertically up to the highest point of the curve. A base line is based on the average of diastolic tone. The resulting height shows the amount of work which the same amount of antigen-antibody reaction has done in the different segments of the various parts of the intestinal canal, if it is allowed to suppose that antigen put in the bath, was diluted into the 300 ccm of TYRODE solution

evenly. From this assumption one may expect to obtain a shorter rise in the duodenal segments and a higher rise in the lower ileal curve. It is true as will be seen from Table II, that one may see the reverse gradation of the maximum height of contraction of the tonus gradient along the gut. Thus, the segments from the lower ileum inscribe a high big smooth curve, while the segments from the upper part of the small intestine inscribe a steep upstroke and a shorter curve, but often with a long continuous plateau following it (See the last item of Table II).

c. Gradient of the refractory condition in sensitized guinea-pigs.

With regard to the refractory conditions in anaphylactic phenomenon, 22 records are analized in Table III. In 15 cases, the horse dander extract was added to the bath in equal amount at the second dose as at the first one. In 7 cases out of these 15, the bath was not changed after the first addition of antigen. None of these guinea-pigs reacted to the second dose of antigen, when it was added 5–23 minutes after the first dose. In the rest of the 8 cases, the bath was changed with fresh TYRODE solution after the first addition of antigen. In 6 cases, the shock was produced by the second addition of horse dander in the same amount as the first dose In these cases, the bath was renewed within 4–5 minutes after the first dose of horse dander was added. In the rest of 2 cases, no reaction was produced by the second addition of antigen. The bath was changed at the 5 th and at the 10 th minute respectively after the first dose. The renewal of the bath solution has a definite relation in the production of a refractory condition of the muscle. The longer the segments contact with diluted antigenic solution, the more they obtain an antianaphylactic condition. More than 10 minutes of contact is necessary to prevent shock from the second dose of the same amount of antigen. Thus, the more frequent the adding of the small amount of antigen to the bath, with regard to the time factor, it is as pointed out by BESREDKA regarding antigen, the greater becomes the antianaphylactic condition. In 3 cases, Nos. 974, 983 and 984, fresh segments were used. They were allowed to contact with diluted antigenic solution for more than 15 minutes, (21, 16 and 27 minutes respectively) without renewal of the bath solution. 10 to 12 times of the first dose of horse dander

extract were added to the bath. They all reacted again at the second dose.

According to COCA and KOSAKAI (26), after partial desensitization, it is necessary to administer 12·5–75 times the amount of the first dose of antigen in order to produce shock in the second trial. WEIL is of the opinion that antigen in the cell modifies the reactivity of the antibody and prevents the shock. With regard to the process of desensitization, either partial or complete, an important factor is the introduction of antigen into the cell itself. WALZER and GROVE (27) conclude from their studies on the properties of precipitins in sensitization using the DALE bath as follows: "If the reaction between antigen and antibody is allowed to complete itself in the bath for which 15 minutes seems to be more than sufficient time, the addition of the same amount of antigen to the bath does not elicit another contraction from the same muscle. The sufficient addition of very much larger amounts of antigen results in a maximal contraction provided the additional antibodies were not completely used up by the first contraction. It may be stated that, if all precipitins are not neutralized in the tissues, as a result of the initial anaphylactic reaction, the introduction of 100 times the original amount of antigen will result in another definite anaphylactic response. It is possible that the factor may be smaller than here stated, smaller amounts may cause increased irritability of the muscle or occasionally a slight contraction. The rule of COCA and KOSAKAI is applicable to precipitins in isolated tissues as studied by the DALE method as well as to precipitins in vitro, and establishes another link in the chain of evidence that precipitins are the antibodies operative in protein anaphylaxis."

As regards the process of desensitization, all of the principles above mentioned have thus proved to be true in my experiments on the sensitized guinea-pig's intestine, using the horse dander extract as antigen, with the exception that COCA and KOSAKAI's factor was found to be less marked in my case.

In considering the gradation of the anti-anaphylactic condition along the intestinal canal, we may see from Table III that the duodenal segments never gave a reaction when the segments from the lower part of the intestine were not able to respond to the second dose; and further that segments from the lower part of the intestine gave a reaction even when the duodenal segments failed to respond.

Namely, the refractory condition is more marked in the duodenum than it is in the lower part of the small intestine. Shall we interprete this phenomenon to the effect that the upper part of the intestine is easier to desensitize than the lower? This anti-anaphylactic gradation, I am inclined to believe at the present time, is based on the tonic gradient.

d. Observations on the preserved intestinal segments (Table II Part B).

Using the intestine preserved in TYRODE solution in an ice-box for several days, the following interesting phenomena are witnessed. 1) The longer the intestine is preserved, the more it becomes soft and lengthens; *i. e.* there is a decrease of tonus. When the tonus is lost completely, then the death of tissue results. By measuring the length of the small intestine of guinea-pig No. 974, it was found to be 85 cm when it was cut from the body and 119 cm after it was preserved in the ice-box for 24 hours. 2) When the segments are set in the bath, the rhythmic contractions start in the segment from the upper part of the intestine. By a careful observation, it will be seen that the orad end of the segment starts to move, first back and forth, then up and down. The segments from the lower intestine take a longer time than those from the upper part before they start the rhythmic contraction. 3) The length of segments shortens more in the upper one than in the lower one. The longer is the period of preservation, the more the segment shortens itself. 4) Guinea-pig No. 953 was tested at 20, 44 and 68 hours after the intestine was taken out. In this we may see that the reactivity is lost from the lower segment while the upper part of the ileum retains its reactivity longer. Since the upper segment has a more potential tonus, it preserves its reactivity longer than the lower segment. Accordingly, for making the anaphylactic test of the ice-box preserved intestine, it is better and safer to use the upper part of the gut, while with fresh specimens, the lower segments show most marked curves and the response is quicker. 5) When the preserved intestine is used in anaphylactic test, a sufficient amount of antigen should be added to the bath. Guinea-pig No. 968 gave a marked reaction with 0·03 ccm (1; 1) horse dander extract in its fresh segment, while the same amount of antigen did not elicit the reaction in the 24 hours old segments,

although the rhythmic contraction in those preserved segments was just as vigorous as in the fresh segments, so far as their amplitude and frequency were concerned. Guinea-pig No. 953, with the 68 hours old segments, the middle ileum gave a marked reaction with 0·1 ccm horse dander extract, but the lower ileum gave no rhythmic contraction even it was in the bath for more than 2 hours. Whenever, there is a rhythmic contraction, an anaphylactic reaction can be obtained by a sufficient amount of antigen. Guinea-pig No. 975 gave no contraction after 3 hours in the bath, consequently, there was no response to antigen. ALVAREZ says that the intestinal muscle usually maintains its power of rhythmic contraction for 5 days. In the above experiments, perhaps I should have waited longer for the return of rhythmic contraction. However, the practical importance of the test lies on the test done on the second or the third day after killing.

e. Effect of change in bath condition.

(Fig. XI) Oxygen was supplied continuously for 6 minutes. There was no change in the character of the curve. It was then stopped for 5 seconds without any change in the curve. It was started again at a rate of 40 bubbles per minute. The curve immediately fell about 17 millimeters in the lower ileum, but to a lesser extent in the duodenum. By the regulation of oxygen I noticed, whenever the bubbles start again after the stoppage, this reaction is always followed. When a change in the rate of oxygen bubbles is made by increasing it suddenly, then the effect on the curve is more marked (Fig. XII and XIII). Often the ascending slope was followed by a higher tonus than before oxygen was increased.

The above mentioned curves were of the sensitized guinea-pigs. In the normal intestine, a similar reaction occurs but it is by no means so marked as the sensitized one (Fig. XIV). A change in the rate of oxygen bubbles seems to have a more marked influence on the sensitized intestine than on the normal intestine. Whether this is due to the mechanical effect of the oxygen bubbles, stirring up the bath suddenly, or to some other causes, I am at present not ready to state. I tried to see the effect of air bubbles by introducing them into the bath from the oxygen outlet. There was a slight effect on the curve, but it was by no means so marked as the effect produced by oxygen bubbles. This would suggest a physico-chemical reaction.

f. Gradient of the circular muscle contraction of a normal guinea-pig's intestine.

Only 11 curves were analyzed for the circular muscle contraction (Table IV). Therefore, I do not like to draw any conclusions from them. A few suggestions I got from their experiments are as follows:

1. A lower hydrostatic pressure is sufficient to elicit a circular muscle contraction in the duodenal segments than in the segments of the lower intestine. In the upper part of the intestine, the circular muscle has a greater tonus than the lower segments have, therefore, it does not expand on increasing the intra-intestinal pressure so readily as do the lower segments where the tonus is less.
2. The gradient of rate of the circular muscle contraction is similar to that of the longitudinal muscle.
3. The gradient of amplitude of the circular muscle contraction is seen clearly: *i. e.* there is a higher amplitude in the upper than the lower segments. By amplitude, in this case, is meant the strength of the segment to push its contents downward by the circular muscle contraction.

By gradually increasing the intra-intestinal pressure of the segment, it becomes shorter and thicker. Whether this shortening is due to the active increase in longitudinal muscle tonus as TRENDELENBURG interpretes it, or a mere passive expansion by the increasing intra-intestinal pressure, it cannot be shown at my present experiments. When the shortening of the longitudinal muscle reached its maximum and the expansion of the circular muscle its maximum allowance point, then the circular muscle contraction starts from the orad end of the segment to the caudad end, pushing the contents out. By the circular muscle contraction, the longitudinal muscle is lengthened passively. The segment becomes long and thin. At the next moment, however, the segment is filled up again with fluid because of its high hydrostatic pressure, and the circular muscle contraction is induced rhythmically.

3. ANAPHYLACTIC REACTION IN THE SENSITIZED GUINEA-PIG AS DEMONSTRATED BY THE INTESTINAL STRIP METHOD.

a. The effect of adding antigen in the bath on the specific contraction of the intestinal muscle.

1) The latent period. (Table II); In most cases, the latent period is about 20–40 seconds. It does not differ from the amount of antigen added to the bath. For example, in the case of guinea-pig No. 977, the ileal segment reacted to 0·015 (1; 1) ccm of horse dander extract in 28 seconds, while in the case of No. 953, the ileal segment reacted to 0·1 ccm of horse dander extract in 35 seconds. According to the "Alles oder Nichts Gesetz", if the given amount of antigen is more than that of the minimum shock producing amount, the muscle gives the shock regularly, almost independent of the amount of antigen added to the bath.

(2) Description of the curve in anaphylactic shock;

a) A moderate reaction of sensitized muscle to antigen···(Fig. I) During the latent period which extends from the addition of horse dander extract to the rise in the curve (48 seconds for the duodenum, 61 seconds for the lower ileum), no marked change of amplitude or frequency occurs. After this latent period, tonus increased gradually, allowing the longitudinal muscle to contract on its upward slope. Then at a certain point of increased tonus of the longitudinal muscle, appears contraction of the circular muscle, as indicated by abrupt fall in the curve in consequence of elongation of the segment and by visualization of mucus being expelled from the segment. These 2 kinds of contraction occur in a stepwise manner in the ascending curve as the muscle tonus increases. After this, the circular muscle contraction decreases gradually in its amplitude and frequency. (Fig. II) During the latent period, no marked change occurs in the rate or in the amplitude of the longitudinal muscle contraction. In the duodenal segment, a marked shock curve results. In the lower ileum, tonus also increases rapidly reaching a plateau where it remained for 50 seconds. Then it rises gradually to its highest point about 20 seconds later. The grade of the tonus increase of the lower ileum is greater than that of the duodenum which indicates that the lower ileal tone is less than the duodenal tone. There are at least 4 slight circular muscle contractions during the period of the plateau. The change of rate and amplitude of the longitudinal muscle contraction is very slight. (Fig. III) The ileal segment alone responds typically to antigen, drawing a big gently ascending slope with several circular muscle contractions on it, resulting in

a zig-zag curve. The duodenal segment does not react typically. The change in the latent period is less marked. The subsequent rise is slight and very gradual. The rate is more rapid and the amplitude is lower than those before the addition of antigen. (Fig. IV) The upper duodenal segment is thrown into tetany at the 66 th second after the addition of antigen, then about 80 seconds later the circular contraction occurs.

In general, the circular muscle contraction was observed to be more marked in the duodenal and the upper ileal segments than in the lower segments. (This is based on the tonus gradient of the circular muscle.) It was also observed that at the time of participation of the circular muscle during the shock reaction, yellowish sticky mucus was pushed out of the segment.

b) A marked reaction of sensitized muscle to antigen···(Fig. V) The latent period is about the same in its duration and character as it is in those of the moderate reaction. After this period, all of the segments are abruptly thrown into tetany, the lower ileum first, the upper ileum next, and the duodenum last, showing a typical gradient in reaction. After the segments reached the highest point in the tetanic reaction, as shown in Fig. IV, irregular contractions with participation of the circular muscle contraction begin, keeping a continuous high plateau. (Fig. VI) In both of the upper (duodenum) and the lower (lower ileum) segments, the up-strokes are interrupted by several zig-zag waves due to the circular muscle contraction. However, the tonus increase in the longitudinal muscle overcomes the circular muscle contraction. Thus, finally, the longitudinal muscle reaches its maximal tonus point. The circular muscle contraction is clearly shown in the duodenal curve, at intervals of 20–60 seconds, About 20 minutes after the addition of the horse dander extract, the circular muscle contraction disappears gradually during the high tonus period. In the ileal segment, a marked tetanic condition makes the curve smooth.

(3) The degree of sensitiveness of the sensitized intestine in the bath: If one considers that in each instance when any amount of horse dander is dropped into the bath, it is actually diluted by 300 ccm of TYRODE solution (all the calculations were based on this assumption), one will realize the delicacy of the reaction. The

minimum shock producing amount of horse dander extract was found in guinea-pig No. 950 (Table I Group I), to be 0·03 ccm of a 1; 100 solution added to 300 ccm of TYRODE's solution, making the final dilution of antigen one to one million. (Fig. VII) With a more diluted antigen, the test was not carried on. For the ordinary test dose, however, 0·05–0·1 ccm of the original horse dander extract added to the bath, making a dilution of 1: 6 000–1; 3 000, seems to be convenient.

(4) The specificity of reaction: The following reactions are sufficient to prove the specificity of sensitization of the intestinal muscle to the horse dander extract. 1) No reaction of the normal intestine to the horse dander up to 3 ccm in the bath occurred. 2) With horse serum the sensitized intestine did not react to the horse dander and the sensitized intestine with horse dander failed to react to horse serum. 3) Guinea-pigs Nos. 961, 1001 and 1002 (Table I Group 2), fed with the horse dander without bile, failed to react to the horse dander.

The facts that a normal intestine gives no reaction to horse dander and sensitized one reacts to 0·0003 ccm of the extract added to the bath, convince that the reaction is one of the true anaphylactic phenomena.

b. Process of desensitization—The anti-anaphylactic condition.

Table III and its following discussion will be found in Chapter 2 under the gradient of the refractory condition in sensitized guinea-pigs.

c. Observation on the comparative degree of sensitization and desensitization of guinea-pigs by the oral and the parenteral methods. (Table I Group 2 and Table III Group 2).

Observation of the kymograph records of shock reaction, does not show any difference between sensitized intestines either by the oral or the parenteral methods. All 3 guinea-pigs gave a positive result to active sensitization by mouth with bile and horse dander. In other 3 used as controls, they gave a negative result to active sensitization to horse dander without bile by mouth. The above reaction shows that sensitization of the guinea-pig body tissue can easily occur if the superficial layers of the gastro-intestinal mucous are laid bare by the action of heterologous ox-bile.

4. EXPERIMENTS ON "ENDOTHELIAL BLOCKADE" ON THE ERGIN CONTENT OF THE INTESTINAL MUSCULATURE.

Trypan blue, India ink and Collargol were used for vital staining for the purpose of the blockade.

1) Trypan blue experiment. (Table I Group 4-a, 4-b and 4-c).

According to EVANS' and SCOTT's findings, I used trypan blue injection in an acute high dosage, and in a prolonged low dosage. After the active sensitization was obtained, trypan blue injection was started (Group 4-a). There was no question about the saturation of the reticulo-endothelial cells in my guinea-pigs. A careful autopsy showed not only a deep coloring of the tissues in which the reticulo-endothelial cells were prominent, but also the coloring of practically all of the other tissues. The urine was blue. In all of the guinea-pigs thus treated, the intestine reacted to antigen in the bath, even to very minute amounts. Namely, the filling or partial filling of the segregation apparatus of the reticulo-endothelial cells by trypan blue had no effect on the actively acquired sensitized condition in my series of 17 guinea-pigs.

In the light of the work of HAENDEL (32, 33), my results are rather discouraging, as I was unable to verify his results. However, the following points should be considered.

(1) The sensitization was done actively.
(2) The Trypan blue injection was given after the actual sensitized condition was acquired.
(3) The most sensitive method to detect the anaphylactic reaction, *i. e.* the isolated smooth muscle test was used.

The last two factors were investigated purposely from the practical standpoint, to find if there is any possibility to utilize the principle of blockade in the treatment of hayfever and asthma as suggested by Dr. COWIE. HAENDEL's experiments on the prevention of horse serum shock in the guinea-pig using India ink, if they can be confirmed, would show very logically the relation between reticulo-endothelial system and anaphylactic shock. Because of this, further investigations in this line should be carried out.

2) Experiments with India ink and Collargol (Table I, Group 4-d and 4-e).

Method of vital staining was carried on according to REIMANN's directions (34). Group 4-d....The result was the same as with trypan

blue. There was no difference in reaction between the treated and non-treated sensitized intestines. I was unable to produce any blockade to the outblow of antibodies from the reticulo-endothelial system or to prevent the development of antibodies by the use of trypan blue and India ink. Group 4-e....All 4 guinea-pigs died from pneumonia. It is doubtful whether the Collargol injection lowered their resistance against the infectious disease. The man who is in charge of the animal house told me that this season (March in Michigan) is the worst time for guinea-pigs against pneumonia.

We must consider the facts that the intestinal muscle where it contains no reticulo-endothelial cells is already sensitized and that blockading the reticulo-endothelial system of cells could probably have nothing to do with desensitizing a remote tissue. My experiment would seem to prove that if we assume the system can be effectively blocked, the tissue once sensitized does not require a continuous supply of sensitizing substance to keep its reaction. I had two guinea-pigs in my series which I injected continuously with trypan blue for 3 months after an injection of active sensitization and still the intestinal muscle reacted to antigen. (Guinea-pigs Nos. 984 and 985) However, the following fact should also be considered. In group 4-c, by the sensitization after a practical saturation of reticulo-endothelial cells with trypan blue, it also happened to induce a positive test by the intestinal strip method. It is hard to tell whether this is due to the partial saturation of the dye-stuff in the reticulo-endothelial cells, or the saturation does not block its function because of its extraordinary power of regeneration (PINEY, 35).

5. SUMMARY

1. Guinea-pigs were sensitized with horse dander extract and the sensitiveness of the guinea-pigs was tested in the so-called DALE bath using intestinal segments. The reasons why I used horse dander extract as antigen and the intestinal strip method as a test of sensitiveness will be found in the discussion about the method.

1. There are physiological gradients (rate and amplitude) in the contraction of a normal guinea-pig's intestine, as were first demonstrated chiefly by ALVAREZ and his associates and were confirmed by COWIE, PARSONS and LASHMET in a normal rabbit's intestine.

By a sensitized guinea-pig, these normal gradients are somewhat

affected as a result of having smaller tonus in the upper part of small intestine than those by a normal one. It means, the sensitized guinea-pig is affected more in the upper part than the lower portion of the small intestine.

1. The specificity of the intestinal strip method in sensitization was proved. The minimum shock producing amount of horse dander extract in my series of experiments was 0·03 ccm of a 1 : 100 solution added to 300 ccm of the bath, making a final dilution of the antigen of one to one million. The delicacy and regularity of the reaction of the intestinal strip would be satisfactory *for the practical use of sensitization test* and I might have a preference for the intestinal strip method to the uterine method.

1. Gradient of latent period in the sensitized intestine, by the influence of antigen, is the reverse of that found in normal guinea-pigs by ALVAREZ. That is a longer latent period in the upper part than that of the lower intestine. The shock curve, we notice that the upper part of the intestine inscribes steeper upstroke and shorter curves than the lower part. The basic reason for these phenomena would be better explained by the tonus gradient of the intestine, than by the metabolic rate of the muscle as ALVAREZ' observation on a normal guinea-pig.

1. Refractory condition is more marked in the upper part than in the lower part of the small intestine. This phenomenon is also based on the tonus gradient.

1. Preserved intestine loses its reactivity from the lower part. It is based on the potential tonus gradient from the upper part to the lower portion of the intestine. Therefore, for the anaphylactic shock test of the preserved intestine, it is recommended to use the upper part of the intestine and to add a sufficient amount of antigen and to use strips not older than the 3 rd day in preservation.

1. Analysis of the curves of shock reaction:
    1. Duration of latent period does not differ from the amount of antigen added to the bath or from the grade of the following shock reaction.
    2. No marked change occurs in the rate or in the amplitude of contraction in the latent period.
    3. During the shock reaction, the tonus increases very rapidly and both the longitudinal and the circular muscle contractions will be seen in the up-hill and at the top of the

curve, making it a zig-zag line, and by a marked reaction the contraction is thrown into a tetanic condition.

4. It gives no difference in the character of reaction of the sensitized intestines between the oral and the parenteral methods.

1. The oral method of sensitization can be accomplished by using heterologous ox-bile as MAKAROFF, ARLOING, LANGERON, and SPASSITCH stated.

1. In the experiments on endothelial blockade my results were rather discouraging. I was unable to verify HAENDEL's results, nor to find any possibility of Dr. COWIE's suggestion.

I wish to express my appreciation to Prof. D. M. COWIE, for the many kind and valuable suggestions and criticism he has given me.

Bibliography had been collected up June 1930, when this work was temporarily finished.

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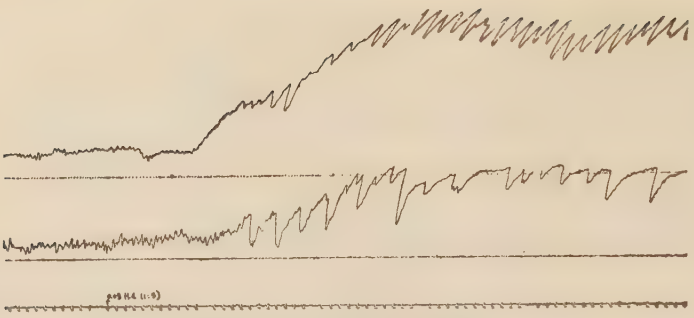

Fig. I. Guinea-pig No. 949.
Upper curve....Duodenum. Lower curve....Middle ileum.

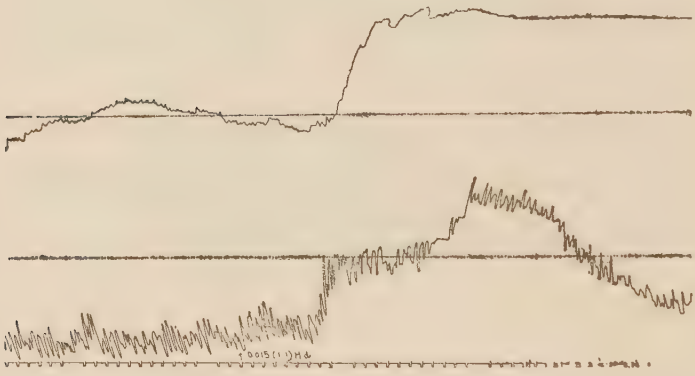

Fig. II. Guinea-pig No. 977.
Upper curve....Duodenum. Lower curve....Lower ileum.

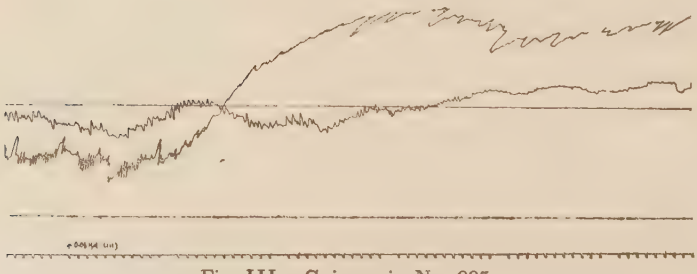

Fig. III. Guinea-pig No. 985.
Upper curve....Duodenum. Lower curve....Lower ileum.

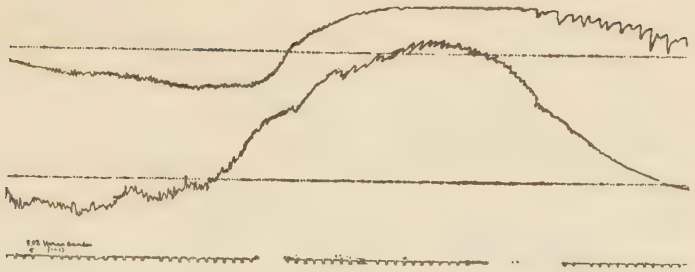

Fig. IV. Guinea-pig No. 980.
Upper curve....Duodenum. Lower curve....Middle ileum.

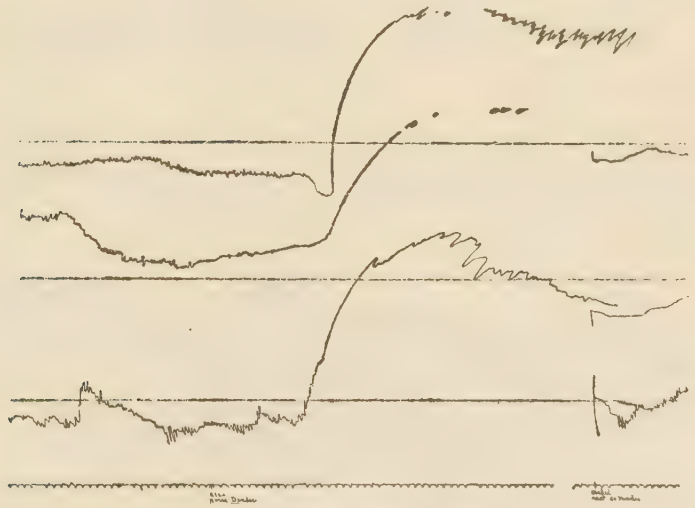

Fig. V. Guinea-pig No. 989.
Upper curve....Duodennm. Middle curve....Middle ileum.
Lower curve....Lower ileum.

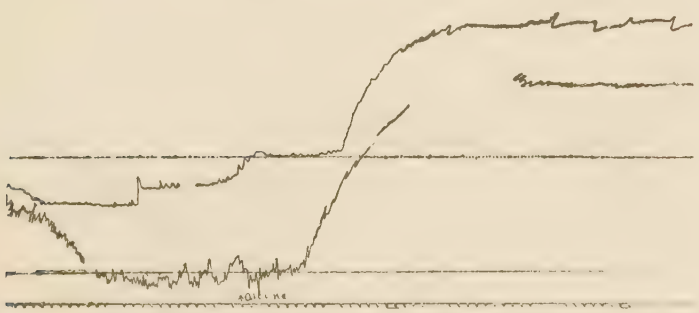

Fig. VI. Guinea-pig No. 953.
Upper curve....Duodenum. Lower curve....Lower ileum.

Table I

Group 1 Sensitization (Parenteral method)

Number of guinea-pig	499	476	485	497	500	990	991	977	473	475	474	472	951	949	947	953	950
Date of sensitization	7-24-'29	7-24-'29	7-24-'29	7-24-'29	7-24-'29	2-11-'30	2-11-'30	2-5-'30	10-13-'29	10-13-'29	10-13-'29	10-13-'29	2-28-'30	2-28-'30	2-28-'30	2-28-'30	2-28-'30
Initial body weight in gram	195	221	220	213	228	382	279	264	207	237	234	210	204	213	198	222	200
Horse dander (i. p.) ccm	2·5	2·5 Twice on successive day		2·5 3-times every other day		2·5	2·5	2·5	1·0 3-times every other day				1·0	1·0	1·0	1·0	1·0
Days elapsed between injection and the bath	49	35		44	28	26	26	31	44	44	37	43	30	28	33	20	47
Date of the bath	9-11-'29	8-29-'29		9-10-'29	8-25-'29	2-5-'30	2-5-'30	3-8-'30	11-26-'29	11-26-'29	11-19-'29	11-25-'29	3-30-'30	3-28-'30	4-2-'30	3-20-'30	4-16-'30
Body weight at the end	325	284		327	352	474	415	407			288	278	273	313	307	306	374
Reaction in the bath	+	+		+	+	±	+	+			+	+		+	+	+	+
Amount of horse dander in the bath (ccm)	0·1	0·1		0·02	0·1	0·015 (1:1)	0·03 (1:1)	0·015			0·1	0·02	0·03 (1:20)	0·03 (1:5)	0·1	0·1	0·03 (1:100)
Remarks		Died on 5th day							Killed by rat								

Table I.

Group 2 Sensitization (Oral method)

Number of guinea-pig	Initial body weight in gram	Sensitization (Antigen with bile)	Days elapsed Between the 1st. administration and bath	Body weight in gram at the end	Date of the bath	Amount of horse dander in the bath	Reaction in the bath	Remark
962	201	Ox-bile 0·5 ccm, then 10-30 minutes later horse dander	24 Days	285	3-5-'30	0·5 ccm	+	
963	173	0·5 ccm by mouth with pipette for 15 days daily	24 „	232	3-5-'30	0·5 „	±	
994	260							Died on 19th day
995	245	The same above for 18 days daily	27 „	306	1-30-'30	0·1 „	+	
996	245							Died on 22nd day
		(Antigen without bile)						
961	222	Horse dander 0·5 ccm by mouth with pipette for 15 days daily	25 „	335	3-6-'30	0·1 „	−	
1001	188	The same above for 16 days daily	26 „	301	1-31-'30	0·1 „	−	
1002	193		33 „	293	2-7-'30	0·1 „	−	

Table I.

Group 3 Desensitization (Repetition of minute doses)

Number of guinea-pig		479	480	488
Body weight in gram		221	156	226
Date of sensitization		9-3-'29	„	9-4-'29
Horse dander ccm (i. p.)		2·5	2·0	2·0
Days elapsed between the 1st. and 2nd Injection		29	„	„
Horse dander extract (1:1600) No symptoms developed for 2 hours after each injection	Intracutaneous 10-20	0·05	„	„
	Intracutaneous 10-6	0·10	„	„
	Intracutaneous 10-9	0·15	„	„
	Subcutaneous 10-13	0·20	„	„
	Subcutaneous 10-15	0·20	„	„
	Subcutaneous 10-18	0·25	„	„
	Subcutaneous 10-22	0·25	„	„
	Subcutaneous 10-25	0·25	„	„
Days elapsed since the 1st. injection		71	72	62
Date of the bath		11-13-'29	11-14-'29	11-14-'29
Body weight at the end		389	383	310
Reaction in the bath		+	+	Technic failure
Amount of horse dander		0·1	0·1	

Table I.

Group 4-a Sensitization with horse dander, then injection with trypan blue

Number of guinea-pig	980	981	982	983	984	985	998	997	976	975	974	973	970	969	968	
Date of sensitization	12–10–'29	12–10–'29	12–10–'29	12–10–'29	12–10–'29	12–10–'29	1–4–'30	1–4–'30	2–5–'30	2–5–'30	2–5–'30	2–5–'30	2–5–'30	2–5–'30	2–5–'30	
Body-weight of guinea-pig	404	338	406	366	326	303	205	220	259	241	198	249	205	261	236	
Sensitization horse dander (i. p.)	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	2·5 ccm	
Days betw. sensitization and trypan blue injection	22	22	22	22	22	22	21	21	30	30	30	30	30	30	30	
Trypan-blue intra-peritoneal injection	(1:1000) 1 ccm daily for 15 days (1:500) 1 ccm every other day for 20 days	(1:1000) 1 ccm daily for 13 days	(1:1000) 1 ccm daily for 15 days (1:500) 1 ccm every other day for 27 days (saturated) 1·5 ccm every 4 days 3 times		(1:1000) 1 ccm daily for 15 (1:500) 1 ccm 17 injections in 27 days then Saturated 1 ccm every 4 days 4 times	Saturated 1 ccm every 4 days 6 times	(1:1000) 1 ccm 3 injections in 6 days (1:500) 1 ccm 6 times in 13 days then Saturated 1 ccm every 4 days 3 times	Saturated 1 ccm every 4 days 2 times	Saturated 1 ccm 3 times during 5 days	Saturated 1 ccm 4 times during 7 days	Saturated 1 ccm 5 times during 13 days	Saturated 1 ccm 8 times	Saturated 1 ccm 6 times during 17 days	Saturated 1 ccm 4 times during 7 days	Saturated 1 ccm 5 times during 11 days	
	Weak solution	Weak solution	Weak then strong	Weak then strong	Weak then strong	Weak then strong	Weak then strong	Weak then strong	strong	strong	strong	strong	strong	strong	strong	
Days elapsed since sensitization	58	35	74	76	80	90	63	51	35	31	49		47	37	41	
Reaction in the bath	+	+	+	+	+	+	+		+	+	+		+	+	+	
Amount of horse dander in the bath	0·03 (1:1)	0·06	0·05	0·03 (1:1)	0·03 (1:1)	0·03 (1:1)	0·03 (1:1)	Killed by rat	0·03 (1:1)	0·03 (1:1)	0·1	Died on 4–4 (Pneumonia)	0·1	0·03 (1:1)	0·03 (1:1)	
Date of the bath	2–6–'30	1–14–'30	2–22–'30	2–24–'30	2–28–'30	3–10–'30	3–8–'30		3–12–'30	3–14–'30	3–26–'30		3–24–'30	3–14–'30	3–18–'30	
Body-weight at the and		396	588	507		477	421		445	376	309		228	388	369	

Group 4-b Trypan-blue and sensitizing injection at the same time

Number of guinea-pig	993	992
Date of sensitization	1–4–'30	1–4–'30
Body-weight of guinea-pig	204	194
Sensitization horse dander (i. p.)	2·5 ccm	2·5 ccm
Trypan-blue intra-peritoneal injection	(1:1000) 1 ccm 23 injections (1:500) 1 ccm 6 injections saturated 2 ccm 1 injection	(1:1000) 1 ccm 23 injections (1:500) 1 ccm 6 injections
Days elapsed since sensitization		41
Reaction in the bath		
Amount of horse dander in the bath	Died on 45 th day (Pneumonia)	–
Date of the bath		2–13–'30
Body-weight at the and		349

Table I.

Group 4-c Trypan blue injection, then sensitization with horse dander

Number of guinea-pig	986	987	988	989	978	979	999	1000
Body weight of guinea-pig	354	448	346	339	201	211	273	266
Trypan blue injection (i. p.)	(1:1000) 1 ccm daily 9 days (1:1000) 1 ccm 15 times in 20 days		(1:1000) 1 ccm daily for 8 days		(1:1000) 1 ccm daily for 3 days		(1:1000) 1 ccm daily for 22 days	
Date of sensitization	1–11–'30	1–11–'30	1–21–'30	1–21–'30	1–7–'30	1–7–'30	1–26–'30	1–26–'30
Sensitization horse dander	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.	2·5 ccm i. p.
Trypan blue injection (i. p.)	(1:1000) 1 ccm 4 injections (1:500) 1 ccm 17 injections saturated 1 ccm 10 injections	(1:1000) 1 ccm 4 injections (1:500) 1 ccm 17 injections saturated 1 ccm 3 injections	(1:1000) 1 ccm 13 injections (1:500) 1 ccm 4 injections	(1:1000) 1 ccm 9 injections	(1:1000) 1 ccm 20 injections (1:500) 1 ccm 6 injections saturated 1 ccm 2 injections	(1:1000) 1 ccm 20 injections (1:500) 1 ccm 6 injections saturated 1 ccm 1 injection	(1:1000) 1 ccm 1 injection (1:500) 1 ccm 6 injections saturated 1 ccm 2 injections	(1:1000) 1 ccm 1 injection (1:500) 1 ccm 6 injections saturated 1 ccm 2 injections
Days elapsed since sensitization	87	58	30	21	48	41	30	30
Reaction in the bath	+	+	+	+		+		
Amount of horse dander in the bath	0·1	0·03 (1:1)	0·05	0·1	Killed by rat	0·1	Killed by rat	Killed by rat
Date of the bath	4–8–'30	3–10–'30	2–20–'30	2–11–'30		2–17–'30		
Body-weight at the end	487	555	394	406		323		

Group 4-d Sensitization, then injection of India ink

Number of guinea-pig	957	958	959	960
Body weight of guinea-pig	230	207	253	235
Date of sensitization	2–28–'30	2–28–'30	2–28–'30	2–28–'30
Sensitization horse dander	1·0 ccm i. p.	1·0 ccm i. p.	1·0 ccm i. p.	1·0 ccm i. p.
Trypan blue injection (i. p.)	* India ink injection (i. p.) 0·5 ccm (1:5) every 2 or 3 days, 9 injections			
Days elapsed since sensitization	†20	20	20	20
Reaction in the bath	+	+	+	+
Amount of horse dander in the bath	0·1	0·1	0·1	0·1
Date of the bath	4–15–'30	4–16–'30	4–15–'30	4–16–'30
Body-weight at the end	411	295	409	398

Group 4-e Sensitization, then injection of collargol

Number of guinea-pig	952	954	955	956
Body weight of guinea-pig	208	202	231	260
Date of sensitization	2–28–'30	2–28–'30	2–28–'30	2–28–'30
Sensitization horse dander	1·0 ccm i. p.	1·0 ccm i. p.	1·0 ccm i. p.	1·0 ccm i. p.
Trypan blue injection (i. p.)	* Collargol injection (i. p.) 0·5 ccm (5 mg) every 2 or 3 days			
Days elapsed since sensitization	20	20	20	20
Reaction in the bath				
Amount of horse dander in the bath	Died on 4–2 after 4 injections	Died on 4–2 after 4 injections	Died on 4–2 after 4 injections	Died on 3–26 after 2 injections
Date of the bath				
Body-weight at the end				

* Collargol and India ink (HIGGINS) were diluted with sterilized distilled water.

† Days elapsed between sensitization and India ink injection or collargol injection.

Table II. Analysis of the curves.

Part a In the fresh segments (Immediately after the guinea-pigs were killed)

Group *		No. 1					No. 2		No. 3		No. 4-a											No. 4-c			
Number of guinea-pig		977	474	472	953	949	995	962	479	480	980	983	982	985	976	984	969	981	970	974	968	979	987	989	988
Amount of horse dander		.015 (1:1)†	0·1	·02	0·1	0·03 (1:5)	0·1	0·5	0·1	0·1	·03 (1:1)	·03 (1:1)	0·1	0·03 (1:1)	0·03 (1:1)	0·03 (1:1)	0 03 (1:1)	0 06	0·1	0·1	0·03 (1:1)	0·1	0·03 (1:1)	0·1	0·05
Latent period in seconds	Duodenum	43	25	20	55	48	73	40	25	60	120	43	35	†	71	37	74	57	42	59	86	33	94	84	43
	Middle-ileum		20	30		71	73		25	95	95		49			36		41			64			73	36
	Lower-ileum	28	18	31	35		40	39	16	54		30		55	15		36	41	30	55		12	85	58	30
Maximum height of contraction in mm	Duodenum	34	50	60		58	57		38	40	32				51		48	62		60	23		33	83	80
	Middle-ileum		65	60		30	90		55	71	62		80					71			67				75
	Lower-ileum	53		83			90		55					62	46		61			60			75	95	71
Time elapsed since contraction started and until its max. height was reacted	Duodenum	30	31	63		93	110		32	45	66				90		76	70		81	43		106	24	44
	Middle-ileum		65	70		83	106		70	10	93		47					93			63				60
	Lower-ileum	70		135			52		44					60	100		69			110			99	27	30
Did the curve come down within 5 min?	Duodenum	No	No	Down		No	No	Down			No	No	No		No	No	No	No	Slowly	No	No		No	No	No
	Middle-ileum		No	Down		No	No				Down		Down			No		No			Down			No	No
	Lower-ileum	Down	No	Down			No	No				Down		No	No		Down	No	Down	No			No	Some-what	No

* Groups are shown in the Table I.
† No marked reaction.

Part b In the old segments (Preserved in ice-box after intestine was removed)

	No. 1			No. 4-a						No. 4-c
	953	953	953	975	974	968	980	982	983	979
Amount of horse dander	0·1	0·1	0·1	0·5	0·1	0·03 (1:1)	0·03 (1:1)	0·05	0·03 (1:1)	0·5
Latent period in seconds										
Duodenum										
Middle-ileum	36	48	43	†	60	†	70	36	100	32
Lower-ileum	20	20	†	†		†	55	28	85	36
Maximum height of contraction in mm										
Duodenum										
Middle-ileum	60	55	28		46		23	33		
Lower-ileum	63	52			97		23	51		
Time elapsed since contraction started and until its max. height was reacted										
Duodenum										
Middle-ileum	78	31	85		55		36	39		
Lower-ileum	60	25			54		25			
Did the curve come down within 5 min?										
Duodenum										
Middle-ileum	No	No	No		No		Down	Down	Down	
Lower-ileum	No	No			No		Sooner	No	Sooner	
Hours elapsed since guinea-pigs were killed	20	44	68	72	28	24	4	2	15	2

† Ratio of the dilution betw. horse dander extract and Coca's extracting fluid.

Table III. Refractory condition.

Part a In the fresh segments

Group	No. 1				No. 2	No. 3		No. 4-a								No. 4-c	
Number of guinea-pig	977	474	472	953	995	480	479	985	980	974	968	970	969	983	984	981	988
Amount of horse dander (ccm) 1 st time	0·015 (1:1)	0·1	0·02	0·1	0·1	0·1	0·1	0·03 (1:1)	0·03 (1:1)	0·1	0·03 (1:1)	0·1	0·03 (1:1)	0·03 (1:1)	0·03	0·06	0·05
Amount of horse dander (ccm) 2 nd time	0·015 (1:1)	0·1	0·02	0·1	0·1	0·1	0·1	0·03 (1:1)	0·03 (1:1)	1·0	0·03 (1:1)	0·1	0·03 (1:1)	0·36 (1:1)	0·36 (1:1)	0·06	0·05
Time between the two doses (min)	7	40	12	23	27	23	29	16	27	21	23	6	5	16	27	23	14
Washed	No	at 5 min	No	No	at 5 min	at 4 min	at 4 min	No	at 10 min	No	at 5 min	No	No	No	No	at 5 min	at 4 min
Reaction Duodenum	−	−	−	−	+	−	+	−	−	−	−	−	−	+	+	+	+
Reaction Middle-ileum		−	−		+	+	+		−		+				+	+	+
Reaction Lower-ileum	−	−	−	−	+	+	−	−		+		−	−	+		+	+
Hours after killing																	

Part b In the old segments

	No. 1	No. 4-a			No. 4-c
	977	974	982	983	979
Amount of horse dander (ccm) 1 st time	0·015 (1:1)	0·1	0·05	0·03 (1:1)	0·5
Amount of horse dander (ccm) 2 nd time	0·1	1·0	1·0	0·03 (1:1)	1·0
Time between the two doses (min)	15	11	19	16	30
Washed	No	No	No	No	No
Reaction	+	−	−	−	−
	+	−	+	−	Slightly +
Hours after killing	2	28	2	15	2

Table IV.
Circular muscle contraction.

Segment	Hydrostatic pressure in mm of tyrode solution necessary to introduce circular muscle contraction				
Duodenum	85	90	88		87
Middle-ileum	90		90	88	90
Lower-ileum	100	95		82	